

SEQUENCE LISTING

<110> Barany, Francis
Zebala, John
Nickerson, Deborah
Kaiser Jr., Robert J.
Hood, Leroy

<120> A THERMOSTABLE LIGASE MEDIATED DNA AMPLIFICATION SYSTEM
FOR THE DETECTION OF GENETIC DISEASES

<130> 19603/3641

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<150> US 08/946,458
<151> 1997-10-07

<150> US 08/462,221
<151> 1995-06-05

<150> US 08/343,785
<151> 1994-11-22

<150> US 07/971,095
<151> 1992-11-02

<150> US 07/518,447
<151> 1990-05-03

<150> 09/480,515
<151> 2000-01-10

<160> 47

<170> PatentIn Ver. 2.1

<210> 1
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<212> DNA
<213> Thermus aquaticus ligase

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aactaccgct actacgtcct ggcggacccg gagatctccg acgcccggta cgaccggctt 180
cttagggagct tcaaggagct tgaggagcgc ttccccgagc tcaaaaagcccc ggactcccc 240

acctttcagg tgggggcgag gccttggag gccacccccc gccccgtccg ccacccacc 300
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<210> 2
 <211> 676
 <212> PRT
 <213> Thermus aquaticus ligase

<400> 2
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Ile Arg Tyr His Asn Tyr Arg Tyr Tyr Val Leu Ala Asp Pro Glu Ile
 20 25 30

Ser Asp Ala Glu Tyr Asp Arg Leu Leu Arg Glu Leu Lys Glu Leu Glu
 35 40 45

Glu Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Leu Gln Val
50 55 60

Gly Ala Arg Pro Leu Glu Ala Thr Phe Arg Pro Val Arg His Pro Thr
65 70 75 80

Arg Met Tyr Ser Leu Asp Asn Ala Phe Asn Leu Asp Glu Leu Lys Ala
85 90 95

Phe Glu Glu Arg Ile Glu Arg Ala Leu Gly Arg Lys Gly Pro Phe Ala
100 105 110

Tyr Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr
115 120 125

Glu Glu Gly Val Leu Val Tyr Gly Ala Thr Arg Gly Asp Gly Glu Val
130 135 140

Gly Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg
145 150 155 160

Arg Leu Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr
165 170 175

Met Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg
180 185 190

Gly Glu Arg Ile Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu
195 200 205

Arg Gln Lys Asp Pro Arg Ile Thr Ala Lys Arg Gly Leu Arg Ala Thr
210 215 220

Phe Tyr Ala Leu Gly Leu Gly Leu Glu Glu Val Glu Arg Glu Gly Val
225 230 235 240

Ala Thr Gln Phe Ala Leu Leu His Trp Leu Lys Glu Lys Gly Phe Pro
245 250 255

Val Glu His Gly Tyr Ala Arg Ala Val Gly Ala Glu Gly Val Glu Ala
260 265 270

Val Tyr Gln Asp Trp Leu Lys Lys Arg Arg Ala Leu Pro Phe Glu Ala
275 280 285

Asp Gly Val Val Val Lys Leu Asp Glu Leu Ala Leu Trp Arg Glu Leu
290 295 300

Gly Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro
305 310 315 320

Ala Glu Glu Lys Glu Thr Arg Leu Leu Asp Val Val Phe Gln Val Gly
325 330 335

Arg Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Leu
340 345 350

Glu Gly Ser Glu Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile
355 360 365

Glu Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala
370 375 380

Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr
385 390 395 400

Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly
405 410 415

His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu
420 425 430

Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Ala Ser Arg Lys
435 440 445

Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu
450 455 460

Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys
465 470 475 480

Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn
485 490 495

Leu Leu Arg Gln Ile Glu Glu Ser Lys Lys Arg Gly Leu Glu Arg Leu
500 505 510

Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn
515 520 525

Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu
530 535 540

Glu Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala
545 550 555 560

Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg
565 570 575

Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu
580 585 590

Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser Arg Pro
595 600 605

Arg Glu Glu Val Lys Ala Leu Leu Arg Arg Leu Gly Ala Lys Val Thr
610 615 620

Asp Ser Val Ser Arg Lys Thr Ser Tyr Leu Val Val Gly Glu Asn Pro
625 630 635 640

Gly Ser Lys Leu Glu Lys Ala Arg Ala Leu Gly Val Pro Thr Leu Thr
645 650 655

Glu Glu Glu Leu Tyr Arg Leu Leu Glu Ala Arg Thr Gly Lys Lys Ala
660 665 670

Glu Glu Leu Val
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<210> 3
<211> 7
<212> PRT
<213> Thermus aquaticus

<400> 3
Asp Ala Glu Tyr Asp Arg Leu
1 5

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerate
Probe

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<221> unsure
<222> (3)

<223> Y at position 3 in this sequence can be either c
or t

<220>
<221> unsure
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<223> Y at position 12 in this sequence can be either c
or t

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or t

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<223> Y at position 19 in this sequence can be either c
or t

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<221> unsure
<222> (6)
<223> N at position 6 in this sequence can be either a,
g, c, or t

<220>
<221> unsure
<222> (18)
<223> N at position 18 in this sequence can be either a,
g, c, or t

<220>
<221> unsure
<222> (9)
<223> R at position 9 in this sequence can be either g
or a

<220>
<221> unsure
<222> (16)
<223> M at position 16 in this sequence can be either c
or a

<400> 4
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<210> 5
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Oligo Primer

<400> 5

agcggataac aatttcacac agga

24

<210> 6

<211> 130

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: pTZ18R

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ccatgattac gaatttaata cgactcacta taggaaattc gagctcgta ccccaaggta 120
cactaggcc 130

<210> 7

<211> 2051

<212> DNA

<213> Thermus aquaticus ligase

<400> 7

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cttagggagc tcaaggagct tgaggagcgc ttccccgagc tcaaaagccc ggactcccc 180
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cgcatgtact ctttgacaa cgccttaac cttgacgagc tcaaggcctt tgaggagcgg 300
atagaacggg ccctggggcg gaagggcccc ttgcgcctaca ccgtggagca caaggtggac 360
ggccttccg tgaacctcta ctacgaggag ggggtcctgg tctacggggc caccgcccgg 420
gacggggagg tgggggagga ggtcacccag aacctcctca ccacccccc catcccgagg 480
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<210> 8

<211> 676

<212> PRT

<213> Thermus aquaticus ligase

<400> 8

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Ser	Asp	Ala	Glu	Tyr	Asp	Arg	Leu	Leu	Arg	Glu	Leu	Lys	Glu	Leu	Glu
35															45

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65															80

Arg	Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe	Asn	Leu	Asp	Glu	Leu	Lys	Ala
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Phe	Glu	Glu	Arg	Ile	Glu	Arg	Ala	Leu	Gly	Arg	Lys	Gly	Pro	Phe	Ala
100															110

Tyr	Thr	Val	Gln	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr
115															125
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130															140
Gly Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg															
145															160
Arg Leu Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr															
165															175
Met Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg															
180															190
Gly Glu Arg Ile Phe Lys Asn Pro Arg Asn Ala Ala Gly Ser Leu															
195															205
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210															220
Phe Tyr Ala Leu Gly Leu Gly Leu Glu Glu Val Glu Arg Glu Gly Val															
225															240
Ala Thr Gln Phe Ala Leu Leu His Trp Leu Lys Glu Lys Gly Phe Pro															
245															255
Val Glu His Gly Tyr Ala Arg Ala Val Gly Ala Glu Gly Val Glu Ala															
260															270
Val Tyr Gln Asp Trp Leu Lys Lys Arg Arg Ala Leu Pro Phe Glu Ala															
275															285
Asp Gly Val Val Val Lys Leu Asp Glu Leu Ala Leu Trp Arg Glu Leu															
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Gly Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro															
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Ala Glu Glu Lys Glu Thr Arg Leu Leu Asp Val Val Phe Gln Val Gly															
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Arg Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Leu															
340															350
Glu Gly Ser Glu Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile															
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Gln Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala
370 375 380

Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr
385 390 395 400

Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly
405 410 415

His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu
420 425 430

Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Ala Ser Arg Lys
435 440 445

Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu
450 455 460

Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys
465 470 475 480

Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn
485 490 495

Leu Leu Arg Gln Ile Glu Glu Ser Lys Lys Arg Gly Leu Glu Arg Leu
500 505 510

Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn
515 520 525

Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu
530 535 540

Glu Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala
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Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg
565 570 575

Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu
580 585 590

Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser Arg Pro
595 600 605

Arg Glu Glu Val Lys Ala Leu Leu Arg Arg Leu Gly Ala Lys Val Thr
610 615 620

Asp Ser Val Ser Ara Lys Thr Ser Tyr Leu Val Val Gly Glu Asn Pro
625 630 635 640

Gly Ser Lys Leu Glu Lys Ala Arg Ala Leu Gly Val Pro Thr Leu Thr
645 650 655

Glu Glu Glu Leu Tyr Arg Leu Leu Glu Ala Arg Thr Gly Lys Lys Ala
660 665 670

Glu Glu Leu Val
675

<210> 9

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 9

ctggcttatac gaaatataat 19

<210> 10

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 10

ccagggcat tttatttct ccatgtacaa at 32

<210> 11

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 11	
catggagaaa ataaaatgac cctggaagag gcg	33
<210> 12	
<211> 18	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence:	
Oligonucleotide	
<400> 12	
aagccggtcg tactcggc	18
<210> 13	
<211> 27	
<212> DNA	
<213> Artificial Sequence	
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<223> Description of Artificial Sequence:	
Oligonucleotide	
<400> 13	
gtttttcatg gtgcacctga cgccctgg	27
<210> 14	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
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<223> Description of Artificial Sequence:	
Oligonucleotide	
<400> 14	
gtttcatgtt gcacctgacg cctct	25
<210> 15	
<211> 23	
<212> DNA	
<213> Artificial Sequence	

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 15
gtcatggtgc acctgacgcc tca 23

<210> 16
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 16
ggagaagtct gccgttactg cc 22

<210> 17
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaA-globin
forward primer

<400> 17
gacaccatgg tgcacctgac tcctgaggag aagtctgccg ttactgccct g 51

<210> 18
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaA-globin
reverse primer

<400> 18
ctgtggtacc acgtggactg aggactcctc ttcagacggc aatgacggga c 51

<210> 19
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 19
tggtaccacg tggactgagg ac

22

<210> 20
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 20
tcctcttcag acggcaatga cgtc

24

<210> 21
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 21
acctcttcag acggcaatcg cgtttc

26

<210> 22
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 22
cccttttcag acggcaatcg cgttttgc

28

<210> 23
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaA-globin

<400> 23
Met Val His Leu Thr Pro Glu Glu Lys Ser Ala Val Thr Ala Leu
1 5 10 15

<210> 24
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaS-globin

<400> 24
Met Val His Leu Thr Pro Val Glu Lys Ser Ala Val Thr Ala Leu
1 5 10 15

<210> 25
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: beta-globin
amplification primer

<400> 25
caacttcatc cacgttcacc ttgcc

25

<210> 26
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: beta-globin
amplification primer

<400> 26
aggcaggag ccagggctgg gg 22

<210> 27
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Alpha1 -
antitrypsin amplification primer

<400> 27
tcagcttac aacgtgtctc tgctt 25

<210> 28
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Alpha1 -
antitrypsin amplification primer

<400> 28
gtatggcctc taaaaacatg gcccc 25

<210> 29
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cystic
fibrosis amplification primer

<400> 29
cagtggaa atggcattct gtt 23

<210> 30

<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cystic fibrosis amplification primer

<400> 30
ggcatgcttt gatgacgctt ctg 23

<210> 31
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaA-globin primer

<400> 31
atggtgccacc tgactcctga 20

<210> 32
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo primer

<400> 32
ggagaagtct gccgttactg 20

<210> 33
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaS-globin primer

<400> 33
atggtgccacc tgactcctgt 20

<210> 34
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Alphal -
antitrypsin(M) primer

<400> 34
ggctgtgctg accatcgacg 20

<210> 35
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo primer

<400> 35
agaaaggac tgaagctgct 20

<210> 36
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Alphal
antitrypsin(Z) primer

<400> 36
ggctgtgctg accatcgaca 20

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cystic
fibrosis (non-508) primer

<400> 37
attaaagaaa atatcatctt 20

<210> 38
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 38
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<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cystic
fibrosis (508) primer

<400> 39
accattaaag aaaatatcat 20

<210> 40
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 40
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<210> 41
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<213> Artificial Sequence

<220>
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Oligonucleotide

<400> 41
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<210> 42
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
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Oligonucleotide

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<210> 43
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<212> DNA
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Oligonucleotide

<400> 43
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<210> 44
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<213> Artificial Sequence

<220>
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Oligonucleotide

<400> 44
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<210> 45
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<213> Artificial Sequence

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<400> 45
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<210> 46
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Oligonucleotide

<400> 46
ggagaagtct gccgttactg cc 22

<210> 47
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
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Oligonucleotide

<400> 47
caggagtcag gtgcaccatg gt 22